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Masafumi Inoue†, Kazuki Tainaka†, Akiyoshi Hirata†, Takashi Konno‡ and Takashi Morii†,*

†Institute of Advanced Energy, Kyoto University, Uji, Kyoto 611-0011, Japan
Fax: 81-774-38-3585, e-mail: t-morii@iae.kyoto-u.ac.jp

‡Department of Molecular Physiology and Biophysics, Faculty of Medical Sciences, University of Fukui, Matsuoka, Eiheiji, Yoshida, Fukui, 910-1193, Japan
Fax: 81-776-61-8101, e-mail: konno@u-fukui.ac.jp.

Post-translational phosphorylation of the microtubule-associated protein tau is believed to have close relevance to the pathogenesis of Alzheimer’s disease. The growth of amyloid fibrils of the short peptide segment VQIVY310K (PHF6) corresponding to the core part of tau fibril formation is strongly affected by the phosphorylation at Tyr310 via its electrostatic pairing with the neighboring charged lysine. Herein, we studied the propensity of resultant fibrils from PHF6 derivative peptides with charged aromatic amino acid residue at Tyr310. The results showed that the phosphorylation at Tyr310 contributed to significant enhancement of the amyloidogenicity and the stability of fibrillar aggregates. The physical origin of remarkable stabilization of fibrils by tyrosine phosphorylation is plausibly attributed to the charge pairing with the adjacent lysine residue in a similar fashion to the phosphorylation effect on the growth of amyloid fibril.

Key words: Amyloid, Fibrils, Phosphorylation, Tau, Aggregates

In Alzheimer disease (AD) one of the primary neuropathological characteristics is the presence of intraneuronal neurofibrillary tangles assembled from paired helical filaments (PHF)[1] comprised of microtubule-associated protein tau in a hyperphosphorylated state.[2,3] Because the development of tau pathology is related to its phosphorylation state, phosphorylation sites on human tau protein have been extensively investigated.[4-13] Some of the phosphorylation sites at serine or threonine residue have been demonstrated to regulate binding to microtubules and fibril formation of tau.[14,15] Three tyrosine residues, Tyr18, Tyr310, and Tyr394, have also characterized as feasible tau phosphorylation sites in Alzheimer’s PHF.[11-13] The Tyr310 is located in the microtubule-binding domain of tau, which is proposed to be a core nucleation site for formation of amyloid fibrils.[16,17] It has been also reported that a short peptide VQIVY310K (PHF6) in the core nucleation site has the greatest amyloid forming potential and the PHF6 peptide and its mutants at the Tyr310 position form amyloid fibrils displaying PHF morphology.[18,19] Because aromatic amino acid residues, Tyr and phenylalanine (Phe), are known to play essential roles in generating and stabilizing the amyloid fibrils, modification at Tyr310 must be effective in altering its amyloid-forming propensity and therefore should have primary importance in biogenesis of amyloid-type fibrils in AD brain. In fact, when a non-natural derivative of tyrosine, such as 4-phenylphenylalanine or 4-methylphenylalanine, is substituted at Tyr310, the aromatic and hydrophobic non-natural amino acids contributed to the significant enhancement of the amyloid-forming propensity.[20] More recently, we have demonstrated that the phosphorylation at Tyr310

Fig. 1. Structures of PHF6 derivative peptides used in this study. Tyrosine at the position X corresponds to the native Tyr310 of tau protein.

strongly influenced the growth of amyloid fibrils of PHF6 due to its electrostatic pairing with the neighboring charged lysine (Lys311).[21] In this article, we investigate the propensity of the resultant fibrils from PHF6 derivative peptides with charged aromatic amino acid residue at Tyr310. The fibrillar aggregates from phosphorylated PHF6 exhibited significantly higher stability to denaturant than those from native PHF6. Phosphorylation effect on the fibril stability can be rationalized by the electrostatic interaction between phosphate group and ε-amino group of Lys311, which is similar to the charge-pairing model of phosphorylation effect on the growth of amyloid fibril.

As previously reported, PHF6 derivatives substituted at the Tyr310 position of PHF6 were synthesized to elucidate a possible effect of charges for the propensity of amyloid fibrils (Fig. 1).[21] The pKa values of the phosphate group of phosphotyrosine are ~2 and 5.8, respectively. It displays one or two negative charges under an acidic or a neutral pH condition, respectively.
The 4-carboxyphenyl group (CF) of PHF6CF reveals a negative charge at neutral pH, but would show no charge under an acidic pH condition. The 4-aminophenyl group (AF) of PHF6AF carries a positive charge under an acidic pH condition, but would show no charge at a neutral pH condition. By assessing aggregation properties of these peptides at different pH conditions, we anticipated that the electrostatic-effect of the phosphate group introduced by the tyrosine phosphorylation for the fibril formation of PHF6 would be clarified.

For TEM analysis, aggregation experiments were conducted in a highly aggregating solution condition (0.5 mM peptide, 20 mM MOPS, 300 mM NaCl, pH 7.5). PHF6, PHF6AF and PHF6CF formed amyloid-type straight or paired-helical fibers (Fig. 2A, C, and D), whereas PHF6pY formed tightly segregated mass of fibers (Fig. 2B) analogous to our previous result.[21] The aggregates had very high contents of β-sheet secondary structure as assessed by FT-IR (Fig. 3). IR spectra of these peptides showed intense amide I bands around 1627 cm⁻¹ characteristic to the β-sheet structure.[22] No high frequency component between 1684 and 1704 cm⁻¹ characteristic to an antiparallel strand configuration was observed. The data suggested that the fibers formed by PHF6, PHF6AF, PHF6pY or PHF6CF contained parallel β-sheet configuration. These fibril properties revealed by TEM and FT-IR measurements are similar to those of fibers formed by the full-length human tau proteins.[23]

CD measurements of PHF6 derivative peptides were performed in the acidic and neutral solutions to evaluate the secondary structure of the peptides immediately after the preparation of aggregates. CD spectra of PHF6 and PHF6AF at pH 7.5 showed intense negative bands between 210 and 220 nm (Fig. 4A), suggesting that both PHF6 and PHF6AF took typical β-sheet configuration. The spectra of PHF6CF and PHF6pY exhibited only weak signal intensities, which was probably caused by strong peptide aggregation and/or light scattering by the aggregates, because of which no proper structural
information could be extracted from the CD spectra of PHF6CF and PHF6pY at pH 7.5. However, the presence of the distinct amide I band characteristic to the β-sheet structure in the IR spectra of PHF6CF and PHF6pY (Fig. 3B and 3D) suggests that the aggregates of the two peptide species that do not contribute for the CD signals took the β-sheet configuration. PHF6, PHF6pY and PHF6CF showed negative bands between 210 and 220 nm in the CD spectra at pH 4.0, whereas PHF6AF showed an intense negative band below 200 nm without showing a negative band between 210 and 220 nm (Fig. 4B). These results indicated that PHF6 and PHF6AF, also possibly PHF6CF and PHF6pY, were predominantly in the β-sheet configuration at the neutral pH condition. At the acidic pH condition, PHF6, PHF6CF and PHF6pY were in the β-sheet configuration, while PHF6AF contained a high fraction of random coil configuration.

Due to the specificity of binding of thioflavin T (ThT) to amyloid fibrils, this dye has been widely used to determine the presence of amyloid fibrils and to examine fibrillation kinetics in situ.[24] In this article, the amount of aggregates formed by PHF6 derivatives was evaluated by ThT fluorescence intensity. ThT fluorescence analysis of these aggregates were performed by 0.1 mM peptide and 10 mM ThT in 20 mM MOPS (pH 7.5) or in 10 mM acetate (pH 4.0) immediately after the preparation of aggregates. ThT fluorescence was measured at the excitation wavelength of 440 nm and emission wavelength of 486 nm. At the acidic pH condition, phosphorylation (PHF6pY) as well as carboxylation (PHF6CF) of the tyrosine residue substantially enhanced the amyloidogenicity of the PHF6 derivative peptides, whereas amination (PHF6AF) hindered the fibril formation at both the acidic and neutral conditions (Fig. 5B).

Stability of the fibrillar aggregates of PHF6 and PHF6pY was next compared quantitatively by monitoring ThT fluorescence at various concentrations of urea, a well-known agent that denature folding and assembly of proteins and peptides. Fibrillization of PHF6 was suppressed at around 1 M urea whereas that of PHF6pY was not affected even in the presence of 2 M urea at pH 7.5 (Fig. 5C). The results demonstrated that the phosphorylation of tyrosine enhanced the stability of the PHF6 fibrils both at the acidic and neutral pH conditions. The carboxyl substitution on the phenyl group increased the stability of fibrils formed by PHF6CF at pH 7.5 (Fig. 5C). On the other hand, the fibril of PHF6CF revealed stability similar to that of PHF6 at pH 4.0 (Fig. 5D), indicating that the anionic carboxylate contributed to enhance the stability of fibrillar aggregates. In contrast, fibrillization of PHF6AF was dramatically suppressed even at 0.5 M urea in the acidic pH condition (Fig. 5D), while the stability of PHF6AF fibrils was comparable to that of PHF6 at pH 7.5 (Fig. 5C). Taken together, the negatively charged group at the tyrosine position, whether it was the phosphate or the carboxylate group, played key roles in stabilizing the resultant aggregates due to the charge paring with the neighboring positively charged residue (Lys 311). Meanwhile, it is likely that the electrostatic repulsion between the positively charged residue of Lys 311 and AF destabilizes the fibril of PHF6AF. These observations were similar to our previous result that the growth of amyloid fibrils of PHF6 is strongly influenced by the phosphorylation at Tyr310 via its electrostatic pairing with the neighboring Lys residue.[21]

In conclusion, comparison of the stability of aggregates
from charged PHF6 derivatives has revealed that the net charge of the peptide has a profound effect on the fibril stability. The electrostatic nature of the phosphate group and its interactions with the neighboring charged lysine strongly enhance the resultant fibril stability. The physical origin of remarkable stabilization of fibrils by tyrosine phosphorylation is plausibly attributed to the charge pairing with the adjacent lysine residue in a similar fashion to the phosphorylation effect on the growth of amyloid fibril.

References


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